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NOTES

Comparative Ultrastructural Study on the Cell Envelopes of *Rickettsia prowazekii, Rickettsia rickettsii,* and *Rickettsia tsutsugamushi*

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Rickettsia tsutsugamushi differs from other rickettsiae in its cell envelope organization. The differences were made evident through a comparative study of the outer envelope of *R. tsutsugamushi, R. prowazekii,* and *R. rickettsii* by electron microscopy.

The basic structural features of rickettsiae as seen by electron microscopy generally resemble those described for other gram-negative bacteria (5, 9). Although numerous descriptions of the fine structure of *Rickettsia prowazekii, R. rickettsii,* and *R. mooseri* (*R. typhi*) have appeared in the literature (1, 2, 7), relatively little information exists on *R. tsutsugamushi* (2). We have found in our current study that, although this organism appears similar to the other rickettsiae in many respects, it differs markedly in the organization of its outer envelope (Fig. 1). The primary purpose of this paper is to compare the physical conformation of the outer envelope of *R. prowazekii, R. rickettsii,* and *R. tsutsugamushi* by electron microscopy.

Chicken embryo (CE) fibroblasts were infected in suspension (C. L. Wisseman and A. D. Waddell, manuscript in preparation) with either the Breinl strain of *R. prowazekii,* the Sheila Smith strain of *R. rickettsii* (both plaque purified), or the Gilliam strain of *R. tsutsugamushi.* The infected cells were grown as monolayers in Falcon flasks at 32°C in an atmosphere of 5% CO_2 in air for 48 to 72 h. The cells were removed from the substrate with trypsin-ethylenediaminetetraacetic acid, centrifuged, and resuspended in 1 to 2 ml of fresh growth medium. Either human convalescent antityphus, human or guinea pig convalescent anti-Rocky Mountain spotted fever, or guinea pig convalescent anti-scrub typhus serum was added to the suspension of infected cells which were then disrupted using 40 strokes of a tight-fitting Dounce homogenizer. The cell suspension was immediately fixed with acrolein-glutaraldehyde followed by osmium tetroxide (5) and embedded in Epon 812 by the method of Luft (8). Thin sections were cut on a Porter-Blum MT-2 ultramicrotome and stained with uranyl acetate and lead citrate. All measurements were made on organisms prepared as above. The structural dimensions of the rickettsial cell membrane, outer envelope, and microcapsular layer have been reported by us (Table 1) and by others (7, 10). A detailed description of the slime layer of rickettsiae appears in another manuscript (D. J. Silverman, et al., Infect. Immun., in press).

The outer envelopes of *R. prowazekii* and *R. rickettsii* appear similar both in overall size as well as in the individual components, i.e., an inner leaflet measuring 6.2 to 7.7 nm, an outer leaflet measuring about 2.5 nm, and a "clear" space between the inner and outer leaflets measuring 2.8 to 4.4 nm. The outer envelope of *R. tsutsugamushi,* on the other hand, although similar in overall dimensions, had an inner leaflet measuring about 2.5 nm and an outer leaflet measuring about 8.5 nm (Fig. 1, Table 1).

Examination of the outer envelopes of *R. prowazekii, R. rickettsii,* and *Escherichia coli* shows that they compare favorably in terms of organizational configuration. Presumably, the peptidoglycan layer of the typhus and spotted fever group of rickettsiae is localized between the outer leaflet of the cytoplasmic membrane and the inner leaflet of the cell wall as in *E. coli.* Occasionally, however, in both the rickettsiae and *E. coli,* this layer appears to be fused with the inner leaflet of the cell wall, giving an increased thickness to the inner leaflet. Because it is the outer leaflet of the cell wall of *R. tsutsugamushi* which is thick when compared with the inner leaflet, the question is raised as to the

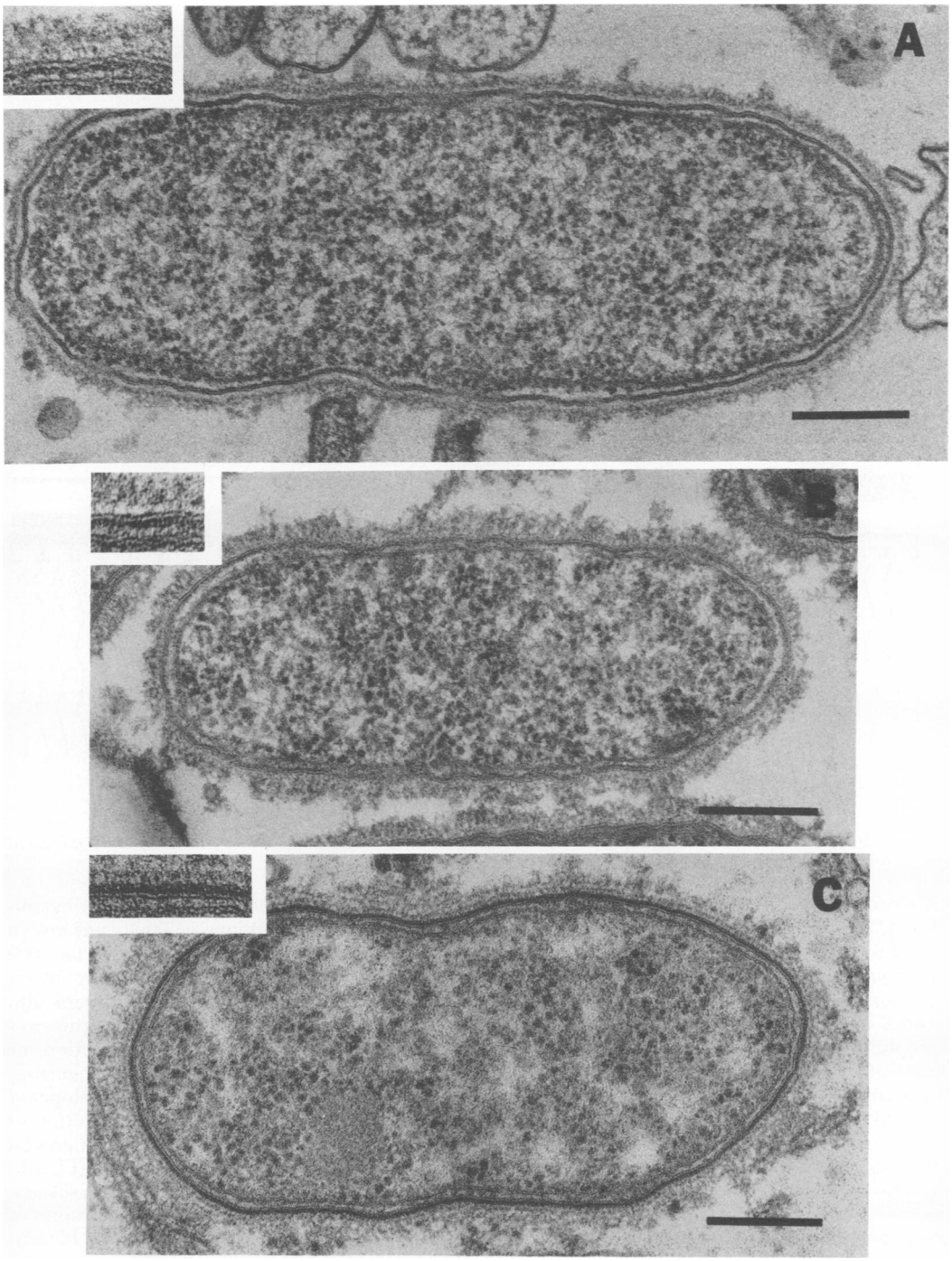

FIG. 1. *Rickettsiae released from infected CE fibroblasts in the presence of specific antiserum. (A) R. prowazekii; (B) R. rickettsii; (C) R. tsutsugamushi. Note thicknesses of inner and outer leaflets of cell wall on (A) and (B) and compare with (C). Bar represents 0.25 μm. Inserts represent approximately fourfold increases in magnification of the same preparations.*

TABLE 1. *Dimensions of rickettsial structures*

Organism	Slime layer (nm)	Microcapsular layer (nm)	Total cell wall (nm)	Inner leaflet[a] (nm)	Clear space (nm)	Outer leaflet (nm)	Cytoplasmic membrane (nm)
R. prowazekii	≤300	16	13	7.7	2.8	2.5	5.5
R. rickettsii	≤125	16	13	6.2	4.4	2.5	5.7
R. mooseri[b]	ND[c]	ND	10	5	3	2	5
R. tsutsugamushi	≤100	14	13	2.5	2	8.5	5.7

[a] Inner leaflet may have a narrow clear space in its center (in *R. tsutsugamushi*, this clear space may occur in the center of the outer leaflet).
[b] Data according to Ito et al. (7).
[c] ND, No data available.

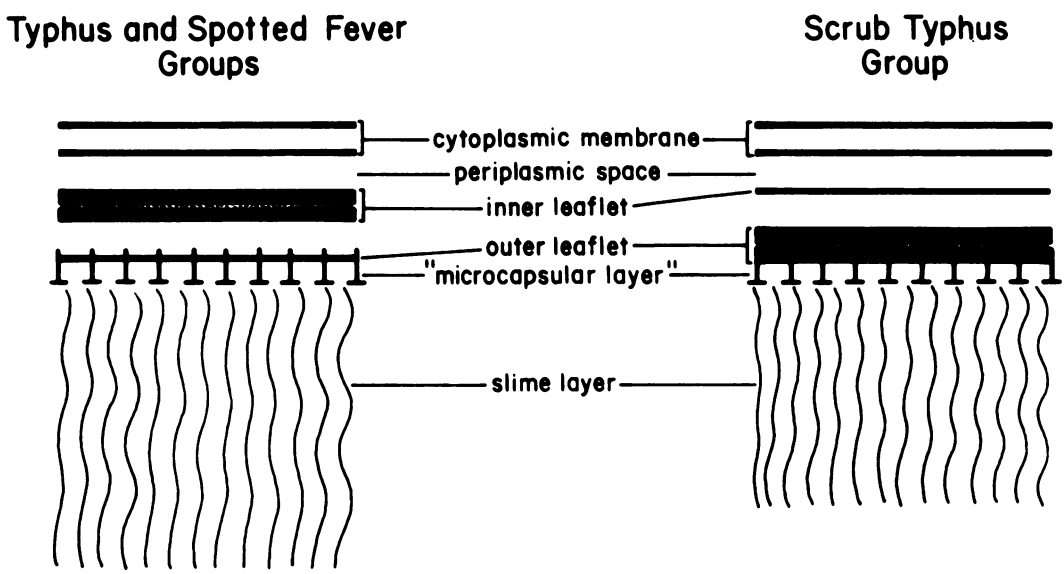

FIG. 2. *Diagrammatic representation of the cell membrane, outer envelope (cell wall), and adjacent extracellular layers of rickettsiae.*

location of the peptidoglycan in this organism.

Treatment of members of the typhus and spotted fever group of organisms with penicillin G (100 μg/ml) for a 24-h period results in the formation of spheroplasts in which the peptidoglycan is no longer evident, either as a separate osmiophilic layer or as an integrally associated portion of the inner leaflet of the cell wall (C. L. Wisseman, Jr., et al., in preparation).

Possibly related to the observed differences in ultrastructural detail between these groups of organisms are the known peculiarities in staining properties of *R. tsutsugamushi* (6) and its resistance to the action of penicillin. Thus, in accordance with earlier findings of others (3, 4), we found that penicillin G (up to 1,000 μg/ml) failed to inhibit growth, judged by comparison of growth rates in the presence or absence of penicillin in slide chamber cultures of infected CE cells by methods previously described (11, 12). Light microscopic examination of such cultures failed to reveal structures resembling spheroplasts, and electron microscopic examination of ultrathin sections of organisms grown in CE cultures in flasks containing 500 to 1,000 μg of penicillin per ml failed to detect any ultrastructural alterations. The basis for penicillin resistance of *R. tsutsugamushi* is unknown—e.g., impermeability, enzymatic destruction, or insensitive peptidoglycan synthetic mechanisms.

In conclusion, the cell wall (outer envelope) of *R. tsutsugamushi* differs markedly from that of members of the typhus and spotted fever groups of rickettsiae in ultrastructural detail (Fig. 2). Differences in staining properties and in susceptibility to penicillin may be manifestations of differences in chemical composition and biosynthetic mechanisms. Further studies on organization, composition, and biosynthesis are indicated.

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